METALS REMOVAL EFFICIENCY AND PHYTOTOXICITY OF MINE DRAINAGE AFTER *CHLORELLA VULGARIS* TREATMENT

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Abstract. The mine drainage with high content of metals and other pollutants has a negative impact on the environment. Phycoremediation using algae as decontamination agents offers an alternative to conventional treatments. In the present study, both the suitability of *Chlorella vulgaris* for mine drainage decontamination was investigated and the removal efficiency of metals ions was also evaluated. The toxicity of mine drainage before and after treatment was assessed by germination experiments using tomato, onion, mustard, beans, sunflower, wheat, and corn seeds. The results revealed that *Chlorella vulgaris* was able to remove high amounts of metals. The highest removal efficiencies were recorded for Pb (90.0 %), Al (93.1 %), Ni (96.6 %), Cr (100.0 %) and Fe (100.0 %). Mine drainage treated with *Chlorella vulgaris* showed a positive effect on germination indices as well, with mean values of germination index (GI) between 67.9 % for tomato and 91.7 % for mustard. Relative seed germination (RSG) for the tested seeds ranged from 80.0 % for tomato to 100.0 % for onion, mustard, beans, sunflower and corn, while Relative root growth (RRG) ranged between 76.5 % for sunflower and 92.5 % for wheat. These results highlighted the suitability of *Chlorella vulgaris* for metals removal from mine drainage with positive effects on seed germination and plant growth (GI > 67.9 %).

Keywords: *Chlorella vulgaris,* mine drainage, toxicological analysis, metals removal

INTRODUCTION

Mining industry has a significant impact on both environmental and socio-economic setting of the world areas. Despite their crucial role in industrial development and growth of economic standards, mining and ore exploitation have generated pollution by releasing dust particles, sludge, heavy metals, toxic effluents and compounds (Krishna Samal et al., 2020). Heavy metals, such as copper, lead, zinc, mercury, cadmium, iron, and nickel are important pollutants from extractive industry (mining, ore preparation, extractive metallurgy). The high concentrations of these heavy metals, the extremely acidic pH and the large amounts of sulfate can cause the contamination of various ecosystems with disastrous consequences in the environment (Rambabu et al., 2020). Their toxicity affects seed germination, roots development and plants growth (Pampuro et al., 2017). Therefore, it is necessary to treat metal-contaminated mine wastes prior to their discharge to the environment (Brar et al., 2022).

Mine drainage is considered the most critical environmental problem created by mining (Kalin et al., 2006). Mine drainage has a perpetual feature due to its leaching capacity and to the activities of various bacteria present in the leachate (Kalin et al., 2006; Bwapwa et al., 2017). Unlike the vast majority of pollutants, most metals do not undergo microbial or chemical degradation (Briffa et al., 2020). Some of the heavy metals salts are soluble in water and cannot be separated by physical methods (El-Zahrani and El-Saied, 2011). Chemical treatment by neutralization, using $CaCO_3$, $Ca(OH)_2$, CaO, Na_2CO_3, NaOH, and NH_3, was widely applied in mining industry. It should be noted that calcium chemicals are cheaper and enable the partial removal of sulfate ions from wastewater (Santos et al., 2020). In the past decades, numerous studies were focused on the development of cheaper and more effective technologies for mine drainage treatment (Sheoran and Sheoran, 2006).

Decontamination of mine drainage using algae as agents of remediation is an advantageous solution due to the low costs, easy manipulation, non-polluting process and simple recovery of the metal contaminants (Kalin et al., 2006; Bwapwa et al., 2017). However, only a few algal species can adapt in the mine drainage conditions to be used for phycoremediation. Phycoremediation is a process applied to remove various pollutants from water using algae (Shackira et al., 2022). Microalgae, such as *Anabaena*, *Chlorella*, *Cladophora*, *Scenedesmus*, *Spirulina*, *Oscillatoria* and *Phaeodactylum* are known for their ability to accumulate heavy metals from mine effluents (Mang and Ntushelo, 2020; Brar et al., 2022). The removal of heavy metals by algae is a flexible process and depends on the metals type, algae specie and environmental conditions (algae are very sensitive to the temperature and light intensity). In the removal process, the metals ions are accumulated in the cell vacuoles and/or intracellular spaces of algae (Bwapwa et al., 2017).

The purpose of this study was to investigate the potential of *Chlorella vulgaris* for mine drainage treatment, concomitantly with the toxicological investigation of mine drainage after treatment with this microalgae specie. The efficiency of metals removal from mine drainage has been also determined.

MATERIALS AND METHODS

1. Mine drainage

The mine drainage (MD) was collected from a mining area, Alba County. Prior to the analysis, the raw MD (RMD) was filtered to remove any unwanted particles. The concentrations of metals in the RMD before treatment are presented in Table 1. The obtained filtrate was used for the treatment with *Chlorella vulgaris*.

2. Mine drainage treatment with *Chlorella vulgaris*

Chlorella vulgaris was inoculated at 1:10 (v/v) ratio in RMD when the exponential phase of growth was reached. Prior to the inoculation, the specie was cultivated in BG-11 as described in our previous paper (Dincă et al., 2020). The cultivation of microalgae in RMD was carried out for 15 days at a temperature of 25 ± 1 °C, under artificial illumination of 1200 lux for 16/8 h day/night cycle, at laboratory scale. After cultivation, the algae biomass was centrifuged at 4000 rpm for 10 minutes and the obtained aqueous extract was used for the germination experiments.

3. Metal analysis

The metal content (Al, Cr, Fe, Ni, Pb Cu, Zn) was measured every 3 days during the 15 days of *Chlorella vulgaris* treatment using an inductively coupled plasma Elan DRC II, quadrupole mass spectrometer (ICP-MS, Perkin Elmer, Canada). The samples were digested with a mixture of 37 % HCl (21 mL) and 69 % HNO_3 (7 mL) and then, diluted to 50 mL. All the reagents used were of analytical grade (Merck, Darmstadt, Germany) and all the dilutions were made with deionized water. Duplicate samples and blanks were used to check precision, whereas accuracy was obtained by using certified standards. Metal removal efficiency (E) was calculated using the following equation:

$$E(\%) = \frac{(C_0 - C_e)}{C_0} \cdot 100 \quad (1)$$

where, C_o and C_e are the initial and final metal content (µg/L).

4. Germination indices

Corn (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), onion (*Allium cepa* L.) and mustard (*Brassica nigra* L.) seeds were considered to investigate the effects of treated MD (TMD) on seed germination. Ten healthy and unbroken seeds were sterilized with sodium hypochlorite (5%) for 10 minutes, washed 5 times with sterile water, and placed between two layers of filter paper (Whatman No.42 mm) in 90 mm Petri dishes. The filter papers were replaced daily to prevent fungal infection. The experiments were performed in triplicate for each set of experiments.

3 mL of the following: distilled water as control (C) (non-biostimulant), raw MD (RMD), and treated MD with *Chlorella vulgaris* (TMD) was added over the seeds. The dishes were maintained at 25 ± 2 °C in dark conditions for 6 days. Germination was considered present and recorded when a visible root appeared on the seed. After 72 h, the length of the roots was also measured (Pampuro et al., 2017). The relative seed germination (RSG) (equation 2), relative root growth (RRG) (equation 3) and germination index (GI) (equation 4) were calculated with the following equations:

$$RSG(\%) = \frac{n_e}{n_c} \cdot 100 \quad (2)$$

$$RRG(\%) = \frac{m_e}{m_c} \cdot 100 \quad (3)$$

$$GI(\%) = \frac{(RSG \times RRG)}{100} \quad (4)$$

where, n_e is the number of seeds germinated after *Chlorella vulgaris* treatment, n_c is the number of seeds germinated in the control, m_e is the mean root length after *Chlorella vulgaris* treatment, m_c is the mean root length in the control (Pampuro et al., 2017).

RESULTS AND DISCUSSION

1. Metal removal

The average removal efficiencies of metals after *Chlorella vulgaris* treatment ranged from 1.8 % on the day 1 (Cu) to 100.0 % (Fe and Cr) on the 15th day of experiment (Table 1). The highest removal efficiencies were observed for Pb, Al, Ni, Cr and Fe, with values of 90.0 %, 93.1 %, 96.6 % and 100 %, respectively after 15 days of treatment. The lowest removal efficiencies were observed for Zn and Cu in the first 3 days. A considerable increase up to day 11 was noted and further the removal efficiencies remained constant for all the metals. The results revealed that *Chlorella vulgaris* was able to hyperaccumulate high amounts of non-essential metals, such as Pb and Al, and essential elements, like Fe, Cu and Zn (Du et al., 2022). Additionally, it was found that algae can also sequestrate Ca, Mg, or Cd in their cells when are exposed to an aquatic environment having a great bioaccumulation potential (Equeenuddin et al., 2021).

Table 1. Metals removal efficiency during *Chlorella* spp. treatment

Metal	Raw MD (µg/L)	E (%)							
		Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
Al	116.6	31.4	65.8	85.2	85.6	86.5	87.5	90.6	93.1
Cr	4.8	42.1	44.7	100.0	100.0	100.0	100.0	100.0	100.0
Fe	51.5	37.4	43.9	100.0	100.0	100.0	100.0	100.0	100.0
Ni	3.6	41.4	75.9	96.6	96.6	96.6	96.6	96.6	96.6
Cu	7.1	1.8	21.1	52.6	61.4	73.7	82.5	84.2	87.7
Zn	10.1	13.6	49.4	61.7	69.1	81.5	82.7	82.7	82.7
Pb	3.8	23.3	63.3	73.3	83.3	90.0	90.0	90.0	90.0

2. Germination indices

The results obtained for RSG, RRG and GI indices are presented in Figures 1-3. The seeds exposed to the TMD showed an improvement in the germination parameters compared to the seeds exposed to MD.

2.1. Effect of *Chlorella vulgaris* treatment on relative seed germination (RSG)

The value of RSG for the tested seeds ranged from 80.0 % (tomato) to 100.0 % (onion, mustard, beans, corn, and sunflower) after 6 days of germination in TMD (Figure 1). The RSG value increased with 10.0 % for tomato, 11.1 % for onion, and 25.0 % for beans exposed to TMD comparatively to the RRG values obtained for RMD. No differences for RSG were observed for mustard, sunflower, wheat and corn when TMD was applied.

2.2 Effect of *Chlorella vulgaris* treatment on relative root growth (RRG)

The RRG values varied between 76.5 % for sunflower and 92.5 % for wheat in TMD (Figure 2). The RRG index increased with 8.2 % for sunflower, 8.3 % for mustard, 10.0 % for onion and 12.1 % for tomato in TMD compared to the RRG values obtained for RMD. Contrary, wheat, and corn seeds marked a low increase of RRG values with 4.3 %, and 6.7 %, respectively. No difference for RSG was observed for beans seeds when TMD was applied.

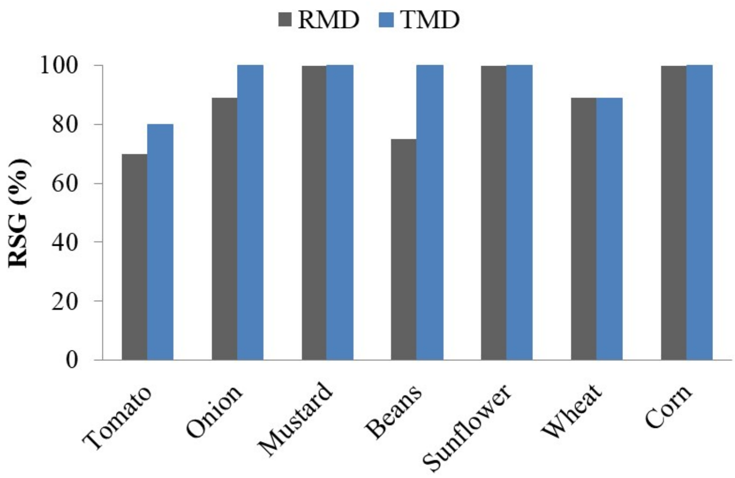

Figure 1. RSG (%) index of seeds in RMD and TMD after 6 days

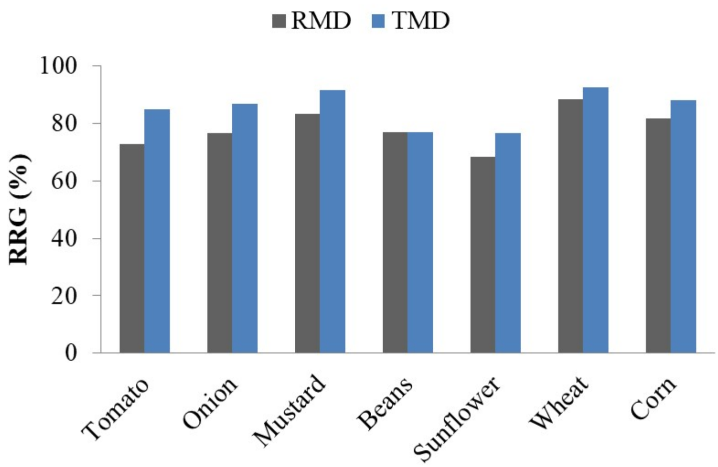

Figure 2. RRG (%) index of seeds in RMD and TMD after 6 days

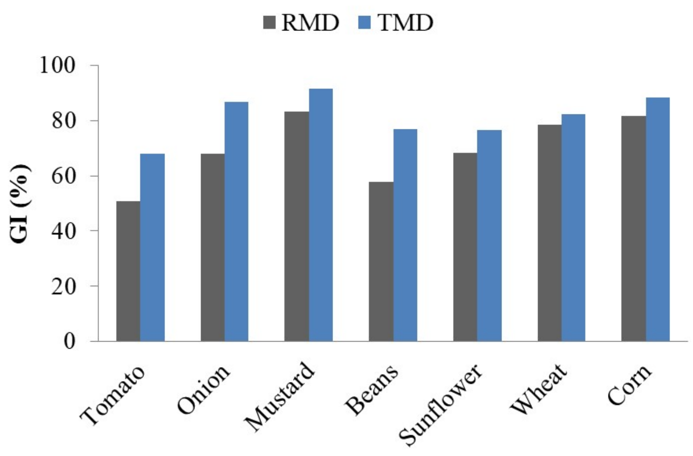

Figure 3. GI (%) of seeds in RMD and TMD after 6 days

2.3. Effect of *Chlorella vulgaris* treatment on germination index (GI)

In TMD, GI varied between 67.9 % for tomato and 91.7 % for mustard (Figure 3). High values of GI were obtained for corn (88.2 %), onion (86.7 %), and wheat (82.2 %). GI increased by 8.2 % for sunflower, 8.3 % for mustard, 17.0 % for tomato, 18.5 % for onion, and 19.2 % for beans after 6 days of germination in TMD compared to the GI values obtained in the RMD. The lowest GI value was recorded for wheat (3.8 %) and corn (6.7 %).

The application of TMD on seeds showed a positive effect on germination indices with increased values of RSG (80.0 – 100.0 %), RRG (76.5 – 92.5 %) and GI (67.9 – 91.7 %) for tomato, beans, wheat and corn. This could be due to the reduction of non-essential elements, which negatively affected the germination mechanisms. It can be concluded that the toxicity of the MD decreased and the potential negative impact on seed germination and plants growth was reduced, taking into account that high metal removal efficiencies and seed germination indices than 90.0 % and 67.9 %, respectively were obtained after 15 days of treatment with *Chlorella vulgaris*. Future investigations will be focus on increasing the GI value above 91.7 % to obtained a toxic free mine drainage.

CONCLUSIONS

The study highlighted the positive effect of mine drainage treatment with *Chlorella vulgaris* in terms of metals removal efficiency. The treatment of mine drainage with *Chlorella vulgaris* proved to be efficient, considering both, metal removal efficiency and toxicological analysis. The results showed high efficiencies for metals removal up to 90.0 %, 93.1 %, 96.6 %, 97.4 % and 100.0 % for Pb, Al, Ni, Cr and Fe, respectively. Germination indices revealed a high reduction of toxicity after treatment with *Chlorella vulgaris* microalgae. The RSG, RRG and GI values increased up to 100.0 %, 92.5 % and 91.7 %, respectively, after MD treatment with *Chlorella vulgaris*. The obtained results suggested that *Chlorella vulgaris* is able to remove high amounts of metals from mine drainage with positive effects on seed germination and plant growth (GI > 67.9 %).

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